Letter to the Editor

Improvement of mustard allergy (*Sinapis alba*) diagnosis and management by linking clinical features and component-resolved approaches

To the Editor:

Approximately 5% of young children and 4% of adults have some type of adverse immune responses to foods in westernized countries.[1] Mustard is a widely consumed spice that can trigger life-threatening IgE-mediated allergic reactions.[2,3] Mustard is frequently consumed as a hidden allergen in foods, resulting in unexpected allergic reactions. It has been reported that more than 50% of patients with mustard allergy are also sensitized to mugwort pollen and various foods of the Rosaceae family, nuts, and legumes.[2,3] Four allergens from yellow mustard seeds have been characterized: (1) Sin a 1 (2S albumin, 14 kd),[4] (2) Sin a 2 (11S globulin, 51 kd),[5] (3) Sin a 3 (lipid transfer protein, 12 kd),[6] and (4) Sin a 4 (profilin, 13-14 kd).[6] Although component-resolved diagnosis of a number of food allergens and aeroallergens has been reported,[7,8] there have not been similar analyses of mustard allergy. In the current study, we combine for first time a detailed clinical characterization of 34 patients with mustard allergy with component-resolved diagnosis by using the 4 purified mustard allergens described so far.

This study was approved by the Fundación Jiménez Díaz Ethic Committee, and written informed consent was obtained from all subjects. Yellow mustard seed extract and purified mustard allergens were obtained as described.[4-6] A skin prick test (SPT) was performed according to standard procedures. A wheal

TABLE I. Clinical characteristics of patients with mustard allergy

		Mustard allergy										
			SPT*			ELISA† OD_{492}						
				Sin a			Sin a					
Patient no.	Sex/age (y)	Symptoms	Mustard	1	2	Mustard	1	2	3	4	Other food allergies	Pollen allergy
1	M/6	OAS, TT (local)	73	91	Neg	1.508	0.738	0.216	Neg	Neg	k,n	No
2	M/18	OAS, U, AE, RC	96	21	Neg	0.267	0.193	Neg	1.118	Neg	r,n,p,l,m	No
3	F/19	U, AE, TT, RC, D	64	25	17	0.113	Neg	Neg	0.879	Neg	r,k,n,p,l	No
4	F/20	OAS, U, AE, RC, D	31	7	Neg	0.228	0.113	0.101	1.061	Neg	r,n,p,l,m	Yes
5	M/23	OAS, U, AE	22	50	Neg	1.196	1.224	0.114	0.606	Neg	r,k,n,p,l	No
6	F/26	OAS (local)	144	43	Neg	0.200	0.117	Neg	0.973	Neg	r,k,n,p,m	Yes
7	F/28	TT, RC	20	Neg	Neg	0.101	Neg	Neg	0.106	Neg	r,k,n	Yes
8	M/29	OAS, U, AE, A	14	12	17	Neg	Neg	Neg	0.110	1.933	r,k,m	Yes
9	M/29	OAS, U, RC, D	87	57	37	2.809	2.648	0.466	1.580	0.857	r,l,m	Yes
10	F/30	OAS (local)	72	69	10	0.347	0.309	Neg	Neg	0.269	k,m	Yes
11	M/30	OAS, AE	11	Neg	Neg	0.232	0.216	Neg	0.899	Neg	No	Yes
12	F/30	OAS, CU (local)	48	Neg	11	Neg	Neg	Neg	Neg	Neg	r,n,p,m	Yes
13	M/30	OAS, U, AE, TT, A, RC, D	103	123	50	1.327	1.153	0.119	Neg	Neg	r,n,p	Yes
14	F/30	OAS, CU, TT, D	119	97	46	3.477	3.532	2.138	Neg	Neg	k,n	Yes
15	M/30	OAS, U, A	163	544	156	2.876	2.345	2.404	Neg	Neg	No	No
16	M/30	OAS, AE, A, RC	169	234	22	0.856	0.643	0.162	0.117	Neg	r,k,n,p,m	Yes
17	M/30	OAS, U, AE, TT, A, RC	171	135	83	0.934	0.920	0.166	Neg	Neg	n	Yes
18	F/31	OAS, U, AE	36	9	13	Neg	Neg	Neg	Neg	Neg	Pineapple	No
19	M/31	OAS (local)	18	ND	ND	0.267	0.162	Neg	0.800	Neg	r,n,p,m	Yes
20	F/31	OAS, AE, CU, TT, A	55	72	25	0.441	0.398	0.111	1.965	0.979	r,k,n,p,m	Yes
21	F/31	OAS, U, AE	68	ND	ND	0.238	0.159	0.101	0.846	0.564	r,k,n,p,m	Yes
22	F/32	OAS (local)	58	9	Neg	Neg	Neg	Neg	0.281	Neg	r,n,p	Yes
23	F/34	OAS, AE, RC	40	Neg	Neg	Neg	Neg	Neg	Neg	Neg	r,n	No
24	M/36	OAS, U	137	353	139	3.500	3.500	2.138	Neg	Neg	r,m	Yes
25	M/36	OAS, AE, A, D	35	47	Neg	0.768	0.584	Neg	Neg	Neg	n	Yes
26	F/38	OAS, AE, TT	23	19	Neg	0.157	0.114	Neg	Neg	0.319	r,k,n,m	Yes
27	F/47	OAS (local)	56	Neg	Neg	0.224	Neg	Neg	Neg	0.163	r,m	Yes
28	M/50	OAS, AE, D	97	108	52	1.041	0.590	0.787	Neg	Neg	No	Yes
29	F/51	OAS, AE, TT	11	Neg	Neg	Neg	Neg	Neg	Neg	Neg	r,k,m	Yes
30	F/51	OAS, U, AE, D	221	170	69	3.366	3.186	0.980	Neg	Neg	No	No
31	F/54	OAS, U, TT, A, RC, D	101	84	35	0.847	0.553	0.529	Neg	Neg	r,k	Yes
32	F/55	OAS, AE, TT	7	Neg	11	0.799	0.468	Neg	Neg	0.175	No	Yes
33	F/68	OAS, U, AE, D	47	66	33	1.274	1.490	Neg	Neg	Neg	n	No
34	F/72	OAS, AE	120	170	29	1.672	1.706	0.221	Neg	Neg	No	No

A, Asthma; *AE,* angioedema; *CU,* contact urticaria; *D,* digestive symptoms; *F,* female; *k,* kiwi; *l,* legumes; *m,* melon; *M,* male; *n,* nuts (including almond); *ND,* not done; *Neg,* negative (<0.100 for ELISA and wheal area <7 mm^2 for SPT); *OAS,* oral allergy syndrome; *p,* peanut; *r,* Rosaceae family (peach, apple, pear, apricot, plum, cherries, and strawberries and excluding almond); *RC,* rhinoconjunctivitis; *TT,* throat tightness; *U,* generalized urticaria.

*Skin prick test: wheal area in mm^2.

†Specific IgE determined in ELISA as OD at 492 nm.

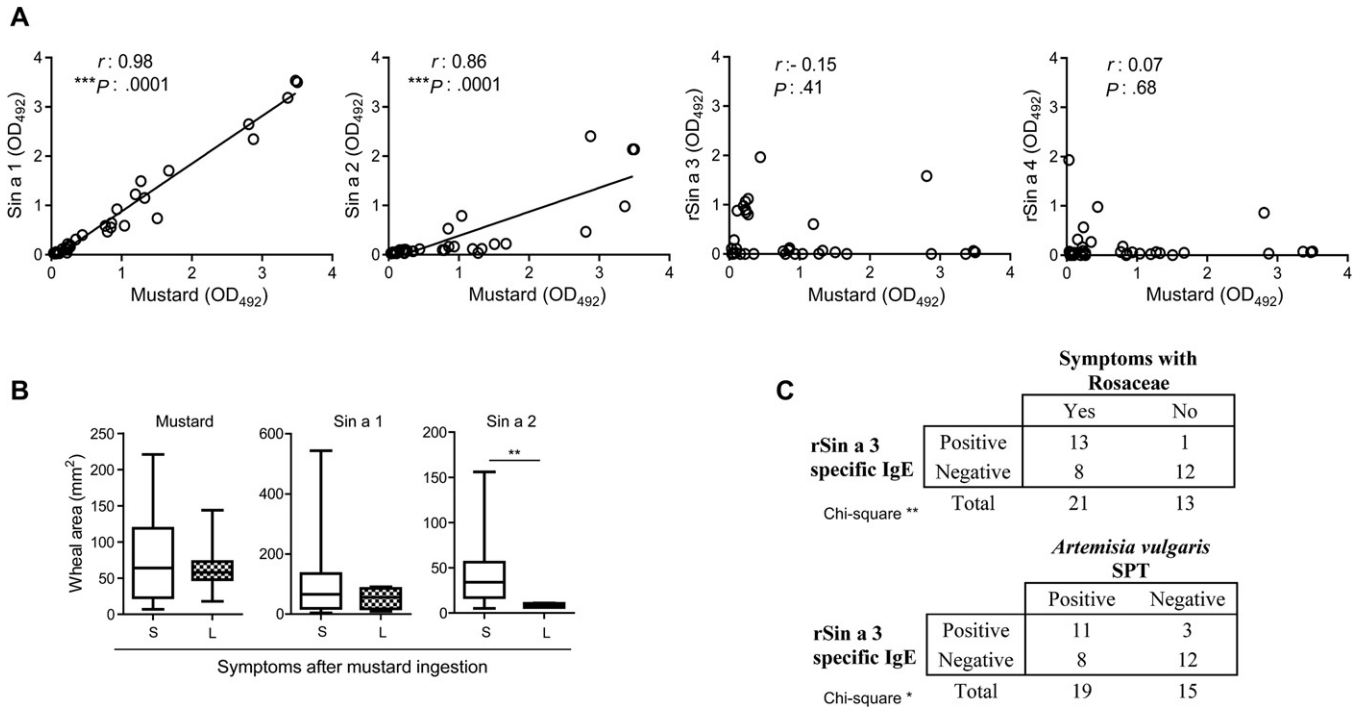

FIG 1. Linking clinical features of patients with mustard allergy with allergen-specific sensitization profiles. **A,** Correlation analysis of the ELISA (OD values at 492 nm) for mustard versus Sin a 1, mustard versus Sin a 2, mustard versus rSin a 3, and mustard versus rSin a 4. **B,** Comparison of the wheal size (in mm^2) induced by mustard, Sin a 1, or Sin a 2 in patients showing systemic *(S)* and local *(L)* reactions after mustard ingestion. $^{**}P < .005$ by Mann-Whitney *U* test. **C,** Analysis of patients showing positive ELISA to rSin a 3 and presenting symptoms with members of the Rosaceae family or showing positive SPT to *A vulgaris* pollen. Significant associations were assessed by χ^2 tests; $^{*}P < .05$, $^{**}P < .005$, and $^{***}P < .0001$.

diameter <3 mm was considered negative. Quantification of specific IgE levels in serum was performed by ELISA. Each OD value was the mean of 2 determinations after blank subtraction. OD values <0.1 were considered negative. The Mann-Whitney *U* test, Spearman correlation, and Pearson χ^2 analysis were used for statistical analysis.

All the patients included in this study reported a clear, immediate IgE-mediated allergic reaction with mustard (within the first 30 minutes) and had positive SPT to mustard extract. Considering that most of the patients reported a positive case history of systemic reactions to mustard and there were technical difficulties in properly masking this spice, no placebo-controlled food challenges to mustard were performed. The main clinical features of these patients (Table I) were comparable to those of other populations of patients with mustard allergy previously reported.[2,3] Twenty-seven patients developed immediate systemic reactions after the ingestion of mustard, 28 had symptoms with other plant foods, and 24 were allergic to pollen. Twenty-five of the 32 tested patients had a positive SPT to Sin a 1 and 19 to Sin a 2. Twenty-five of 34 tested sera had positive IgE to Sin a 1, 16 to Sin a 2, 14 to recombinant (r) Sin a 3, and 8 to rSin a 4 as determined by ELISA.

We found significant positive correlation when comparing SPT and ELISA results for mustard, Sin a 1, and Sin a 2 (data not shown), indicating that both *in vivo* SPT and *in vitro* ELISA determinations can be used to assess sensitization to mustard, Sin a 1, and Sin a 2. Sin a 1 was the most prevalent mustard allergen, and we found a highly significant positive correlation when plotting the specific IgE levels to mustard versus those to Sin a 1 (Fig 1, *A*). A significant positive correlation was also found when comparing the specific IgE levels to mustard and Sin a 2, but not to mustard and rSin a 3 or to mustard and rSin a 4 (Fig 1, *A*). These results indicate that Sin a 1 is the most suitable allergen to be used as a diagnostic marker to determine genuine sensitization to mustard. Patients 7, 11, and 27 had negative SPT to both Sin a 1 and Sin a 2 but showed specific IgE to either rSin a 3 (7 and 11) or rSin a 4 (27). Patients 23 and 29 showed positive SPT to mustard but negative ELISA results to all the assayed allergens, suggesting that these patients might be sensitized to an undiscovered mustard allergen.

When comparing the wheal size induced with mustard extract or Sin a 1 in patients presenting with systemic symptoms with those presenting with exclusively local symptoms, we did not find statistically significant differences (Fig 1, *B*). In contrast, the wheal size of Sin a 2 in the group of patients with systemic symptoms was significantly higher than in the group of patients with only local symptoms (Fig 1, *B*), indicating that SPT to Sin a 2 could be the test to predict severity of symptoms in patients with mustard allergy. The patients in the study with systemic reactions to mustard (n = 27) rarely used self-injectable epinephrine, and half of them (n = 14) sought medical attention in an emergency department where epinephrine was administered. Because most of the reactions to mustard occurred during the first 30 minutes after ingestion, patients with mustard allergy require instructions on the timely use of self-injectable epinephrine.

Our data indicate that among patients with mustard allergy, there is a significant association between having specific IgE to rSin a 3 and having allergy to other plant-derived foods such as Rosaceae fruits ($P < .005$), mainly peach, or *Artemisia vulgaris* pollen ($P < .05$; Fig 1, *C*). These results are in agreement with the involvement of the lipid transfer protein family in cross-reactivity among cabbage, mugwort pollen, and peach previously reported.[9] Although a correlation between the mustard profilin Sin a 4 and cross-reactivity with other foods and pollen was observed, clinical associations involving profilin were not statistically significant, probably because of the limited number of patients sensitized to Sin a 4. Considering the very low amount of profilin in yellow mustard seeds,[6] we suggest that Sin a 4 may not act as a sensitizing allergen but rather as a cross-reactive agent involved in pollen and/or other food allergies.

In conclusion, this work contributes to improve diagnosis and management of mustard allergy by correlating clinical presentations of patients with mustard allergy with their sensitization profiles to specific mustard allergens. We propose that purified Sin a 1 and Sin a 2 mustard allergens should be routinely included in SPT assessment to evaluate primary sensitization to mustard and warn about the severity of the symptoms triggered by mustard consumption, respectively. Specific-IgE determinations to rSin a 3 and rSin a 4 are also recommended to determine potential cross-reactivity involving other plant-derived foods and pollens.

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Andrea Vereda, MD, PhD[a]
Sofía Sirvent, BSc[b]
Mayte Villalba, PhD[b]
Rosalía Rodríguez, PhD[b]
Javier Cuesta-Herranz, MD, PhD[a]
Oscar Palomares, PhD[b]

From [a]Servicio de Alergia, Fundación Jiménez Díaz; and [b]Departamento de Bioquímica y Biología Molecular, Facultad de Química, Universidad Complutense de Madrid, Madrid, Spain. E-mail: oscar.palomares@quim.ucm.es.

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